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Immunohistochemical Subclassification of Peripheral T Cell Lymphomas-Not Otherwise Specified

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Abstract

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Objective Peripheral T cell lymphomas-not otherwise specified (PTCL-NOS) are a heterogeneous group of mature T cell lymphomas that do not belong to any specified subtype. Gene expression profiling has revealed two biological variants of PTCL-NOS, PTCLGATA3 and PTCLTBX21. This study aims to subclassify PTCL-NOS using immunohistochemistry (IHC) and to establish its implication in prognosis.

Methods A descriptive study was done using 39 morphologically and immunohistochemically diagnosed cases of PTCL diagnosed over a 10-year period, 2013 to 2022. The additional IHC markers used were GATA3 and CCR4 for PTCLGATA3 and TBX21 and CXCR3 for PTCLTBX21. All cases had a minimum follow-up of 6 months.

Results After subclassification of PTCL-NOS ($n = 39$), 44% were PTCLGATA3 subtype. This subtype showed monomorphic morphology and a high ki67 index, and was found to a have worse outcome. In comparison, PTCLTBX21 showed heterogeneous morphology and a low ki67 index.

Conclusion Subcategorization of PTCL-NOS based on the IHC markers helps in the stratification of this disease. This will also identify cases with more aggressive behavior at the time of diagnosis.

Keywords

- PTCL
- subclassification
- prognosis
- IHC-GATA-3

Introduction

Non-Hodgkin's lymphomas are neoplasms of lymphoid tissues and are categorized into T cell, B cell, and natural killer (NK) cell lymphomas. T cell lymphomas (TCLs) are a diverse group of neoplastic diseases with aggressive clinical course.[1,2] Currently, molecular and gene expression profiling (GEP) studies and cancer-related mutations have been added to the classification of TCLs to improve their definition and diagnostic criteria.[1,3–5] These neoplasms are more common in Asia compared with America and Europe.[2,6–8] The prevalence pattern of TCLs in India is different from that of the other countries.[9–14]

Peripheral TCL-not otherwise specified (PTCL-NOS) is a complex subtype and a diagnosis of exclusion. Two possible biological variants of PTCL-NOS, PTCLGATA3 and PTCLTBX21, have recently been discovered by studying the transcriptional pathways of T helper-2 (TH2) and T helper-1 (TH1) cells. The transcription factors GATA3 and TBX21 are regulators of TH2 and TH1 differentiation, respectively.

GATA3 causesTH2 cytokine secretion and inhibits gamma interferon production. GATA3 regulates the T cell development and function and NK cell production. It is also involved in tumorigenesis. On the other hand, TBX21 is a TH1-specific transcription factor which causes TH1 gene expression programs. Thus, GATA3 and TBX21 regulate cytokine and

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chemokine productions by the T helper cells. CXCR3 and CCR5 are the chemokines produced by TH1 and CCR4, CCR3, and CXCR7 are chemokines of TH2 cells. Hence, the different TCLs are also found to selectively express these chemokines.

Molecular studies and GEP have discovered subsets in PTCL-NOS, (1) PTCLGATA3, characterized by high expression of GATA3 and its target protein CCR4 and CXCR7, and (2) PTCLTBX21, characterized by high expression of TBX21 and its target protein CXCR3 and CCR5. Molecular techniques being costly and cumbersome, are seldom used in everyday clinical and pathology practice. Use of formalin-fixed paraffin-embedded (FFPE) tissue-based immunohistochemical (IHC) staining can help convert GEP-based diagnostic signatures to a cost-effective user-friendly diagnostic tool, as adapted by Amador et al.[15]

Subclassification of PTCL-NOS study has not yet been conducted in Indian population, as per our literature search, though the incidence of PTCL-NOS is 27.63% of all TCLs in India.[10]

This study aims to subclassify PTCL-NOS based on morphological features and IHC and to identify the significance of this subclassification in prognosis.

Methods

A descriptive study was done including all patients with morphologically and immunohistochemically confirmed diagnosis of PTCL-NOS from January 2013 to June 2022 in our institute. The cases were selected retrospectively (already diagnosed based on IHC) and the new IHC markers for subclassification were then additionally done.

IHC was done in all cases of PTCL-NOS whose blocks were retrievable and had a minimum follow-up of 6 months. Cases with inadequate tissue/follow-up were excluded.

Based on the prevalence of subclasses of TCLs (PTCL-NOS, 62%) observed in an earlier publication[14] and with 20% relative precision and 95% confidence, the minimum sample size was determined to be 59. In this study, 151 TCLs cases are selected, out of which 44 were PTCL-NOS.

Data of all the cases (age, sex, stage, bone marrow involvement, and follow-up) were retrieved from the Amrita Hospital Information System (AHIS) database. All the morphological features and immunohistochemical characteristics were documented.

PTCLs were diagnosed using the panel of IHC including CD3, CD5, CD7, CD4, CD8, CD56, CD30, CD10, BCL6, CD23, CD20, PAX5, EBV-LMP, and ALK.

The additional antibodies selected included: GATA3 and CCR4 for PTCLGATA3, and TBX21 and CXCR3 for PTCLTBX21 in accordance with a previous study.[15]

FFPE blocks of all cases of PTCL-NOS were taken and cut at 3 µm using microtome. Hematoxylin and eosin slides were used to assess the morphology. The tissue sections were processed for antigen retrieval and stained using IHC stains GATA3, CCR4, TBX21, and CXCR3.

- GATA3 - PathNsitu, Mouse monoclonal, L50823
- TBX21 - Brand Cell Signaling Technology, Rabbit monoclonal antibody, D6N8B
- CCR4 - Brand Sigma Aldrich, Rabbit polyclonal, HPA031613
- CXCR3 - Brand Genetex, GTX108145

Assessment of immunohistochemistry expression:

GATA3 - Nuclear (positive- nuclear positivity in $\geq$ 50% of malignant cells)
CCR4 - Membranous (positive- membranous positivity $\geq$ 50% of malignant cells)
TBX21 - Nuclear (positive- nuclear positivity in $\geq$ 20% of malignant cells)
CXCR3 - Membranous (positive- membranous positivity $\geq$ 20% of malignant cells)

Data regarding the lactate dehydrogenase (LDH) levels, international prognostic index (IPI) score, extranodal sites involved, Eastern Cooperative Oncology Group score, relapse/recurrence/progression, and follow-up were obtained from AHIS. Ki67 index, CD4/CD8 status, and CD30 positivity were also recorded.

Statistical analysis was performed using IBM SPSS version 20.0 software. Categorical variables were expressed using frequency and percentage. Continuous variables were presented using mean and standard deviation. Chi-square with Fisher's exact test was used to study the statistical significance of the comparison of all categorical variables between the two groups. To find the best marker of PTCL-NOS to subclassify, diagnostic measures such as sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were calculated. Kaplan–Meier survival analysis was used to study the overall survival (OS) and disease-free survival (DFS) time and log rank test was used to compare the survival time between both the groups. A p-value of < 0.05 was considered to be statistically significant.

Results

After morphological and IHC evaluation, 44 patients (29.1% of TCLs) were found to have PTCL-NOS, which showed an immune profile of CD3 positivity with variable loss of CD5/CD7. Thirty-six cases showed CD4 > CD8. Cells were negative for CD20, PAX-5, CD15, ALK-1, CD10, BCL6, and EBV-LMP. Three cases showed CD30 positive ALK negative cells, which were tested for EBER-ISH and turned out to be negative.

In PTCL-NOS, blocks were retrievable for 39 cases. IHCs were done in these cases and were subclassified into two groups: PTCLGATA3 and PTCLTBX21. Sixteen cases were of GATA3 subtype and the rest, TBX21.

Four IHC markers were used in our study for the subclassification of PTCL-NOS which are "GATA3 and CCR4" in the GATA3 group and "TBX21 and CXCR3" in the TBX21 group. According to a previous study,[15] GATA3 subclass is defined by both/one of GATA3/CCR4 showing positivity in more than 50% cells with less than 20% cells showing positivity for TBX21 and CXCR3. TBX21 subclass is defined as cases with more than 20% cells showing positivity in either/both of

Table 1 Association between each marker and PTCL-NOS subclasses

Marker positive	Subclasses		*p*-Value
	GATA3, 16 (%)	TBX21, 23 (%)	
GATA3 (> 50%)	13 (84.6)	3 (15.4)	< 0.001
CCR4 (> 50%)	14 (91.7)	2 (8.3)	< 0.001
TBX21 (>20%)	1 (6.25)	9 (39)	0.020
CXCR3 (> 20%)	0	23 (100)	< 0.001

Abbreviation: PTCL-NOS, peripheral T cell lymphomas-not otherwise specified.

TBX21/CXCR3 along with less than 50% cells showing positivity for GATA3 and CCR4. GATA3 subclass included 16 (44%) cases and TBX21 subclass included 23 (56%) cases (►Table 1).

After the IHC, the prognostic variables included in the IPI and prognostic index for TCL (PIT) scoring system like age > 60 years, high LDH levels (> 333 IU/L), > 1 extranodal sites involved, Ann Arbor stage III/IV, and high ki67 levels (≥ 80%) were compared between subclasses. No statistical significance was found while comparing age, stage, LDH levels, CD4/CD8 status, CD30 levels, IPI scores, and number of extranodal sites between the subclasses.

Note that 83.3% of PTCLGATA3 showed a monomorphous morphology (►Fig. 1) showing uniform population of medium-sized cells with scanty cytoplasm and cleaved nuclei. In PTCLTBX21, 80% cases were polymorphic (►Fig. 2), showing medium-sized lymphoid cells admixed with plasma cells, eosinophils, and histiocytes in variable proportions. There is a statistical significance between the morphological subclasses with a *p*-value of 0.002.

K_i67 values ranged from 50 to 90% with a mean of 70% in the PTCLGATA3 group. In the other group, the K_i67 values ranged from 25 to 80% with a mean value of 52.5%. Fifty percent of PTCLGATA3 cases showed values equal to or above 80%. In PTCLTBX21, a single case had a ki67 value of 80% (►Table 2).

An event in our study is described as the occurrence of a recurrence, relapse, and disease progression. In PTCLGATA3, three cases had recurrence and one died due to the disease (25%). In PTCLTBX21, three (13.3%) cases had recurrence.

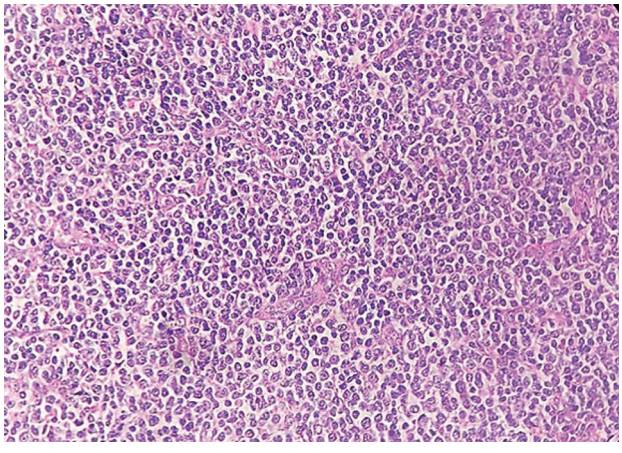

Fig. 1 Peripheral T cell lymphoma (PTCL) showing monomorphous population of neoplastic cells.

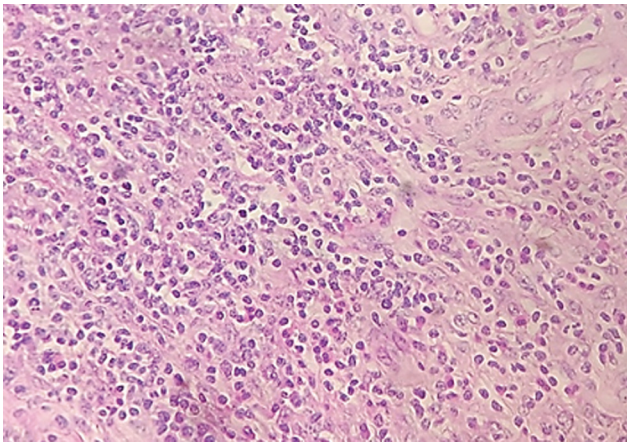

Fig. 2 Peripheral T cell lymphoma (PTCL) showing polymorphous population of neoplastic cells admixed with eosinophils and plasma cells.

Table 2 Comparison of ki67 levels with the subclasses of jPTCL-NOS

	Subclasses		*p*-Value
	TBX21 (*n* = 23) %	GATA3 (*n* = 16) %	
< 80%	16 (95.7)	8 (50)	0.024
≥ 80%	1 (4.3)	8 (50)	

Abbreviation: PTCL-NOS, peripheral T cell lymphomas-not otherwise specified.

Discussion

29.1% of TCLs constituted PTCL-NOS in our study and was the most common type noted. This is in concordance with another study from the same state in India.[10] But this was lower when compared with most of the Indian and western studies.[6,12,14] The median age at diagnosis was high (65.5 year) when compared with other studies.[14] Note that 93.9% patients presented with an advanced stage and 44.8% patients presented with a marrow involvement.

Among the IHC panel for subclassification, it was noted that CXCR3 showed 100% sensitivity, specificity, and accuracy. TBX21 was the least sensitive marker with 40% sensitivity and 66.67% accuracy. In comparison, GATA3 had a sensitivity of 91.67% with a specificity of 86.67% and

accuracy of 88.89%. Additionally, CCR4 demonstrated a sensitivity of 91.67%, specificity of 93.33%, and accuracy of 92.59%. Overall, GATA3, CCR4, and CXCR3 together were the most helpful markers in the subclassification of the cases in our study. TBX21 maker showed a nonspecific staining in some of the cases in our cohort possibly due to technical issues. Overall, only 39% cases categorized as PTCL-TBX21 showed TBX21 marker positivity. In a related study,[1] the use of the TBX21 stain alone resulted in incorrect categorization of TBX21 subclass (69%) as opposed to the use of this stain in conjunction with CXCR3 (88%). This might also account for the lower sensitivity of the TBX21 stain seen in our research.

The percentage of GATA3 (44%) patients were more in our cohort when compared with a previous study conducted by Iqbal et al,[5] which showed 33% to be of GATA3 subtype.

According to previous studies,[5,15,16] GATA3 subclass showed a monotonous population of cells without much inflammation in the background. A similar profile was noted in our study with 83.3% cases composed of predominantly monomorphous cells. The cells were either medium-sized with moderate clear to eosinophilic cytoplasm or large-sized with minimal cytoplasm resembling blastoid cells. Frequent mitosis was noted in all cases.

TBX21 subclass shows a polymorphous population according to literature.[5,15] Similar observations were noted in our study which showed 80% patients showing a polymorphous morphology. Two kinds of polymorphous patterns were noted within the TBX21 subclass. In one, the atypical cells were seen with variable sizes with a background showing mixed inflammation composed of neutrophils, small lymphocytes, eosinophils, histiocytes, and plasma cells. In the other type, also called the Lennert's pattern, the neoplastic cells were intermixed with epithelioid histiocytes.

We found a significant difference (p-value 0.002) between the two morphological patterns (monomorphic vs. polymorphic) associated with the two subclasses in our study.

Ki67 is a nuclear IHC stain and a proliferation marker. According to modified PIT scoring system, a ki67 index $\geq$ 80% is considered high and was associated with poor outcome in PTCLs. A significant difference in terms of ki67 expression was noted between the subclasses in our study ($p = 0.024$). It was seen that a high ki67 value was associated with 50% cases in PTCLGATA3. On the contrary, only one case showed a high ki67 in PTCLTBX21 which can be related to a better outcome.

Thirty-six cases showed a CD4+/CD8– expression pattern, with no difference between the two subgroups. In comparison, a significant difference was noted with a heterogeneous expression of CD4/CD8 levels in the TBX21 subclass in the study by Amador et al.[15] This difference could be due to the lesser number of cases in our cohort. The frequency of CD30+ cases were also similar between subclasses which was similar to other studies.[5,15]

Except for a single (9.1%) case in GATA3 subclass, all cases were stage II or higher, according to the Ann Arbor staging in both the subclasses. The extranodal sites involved in the patients include the bone marrow, lung, pleura, central nervous system, skin, and nasopharynx. All the findings were similar with a comparable study.[15]

The study by Iqbal et al[5] compared the 5-year OS between the two subclasses and found that the TBX21 subclass showed a better survival . The median OS in our study did not reveal any significant difference possibly due to a smaller cohort. However, the mean OS varied between the two subclasses. The TBX21 subclass showed a mean OS of 27.35 months (standard error 9.38) and the GATA3 subclass with 8.4 months (standard error 2.6). The DFS is computed as the time from diagnosis to the occurrence of a recurrence, relapse, progression, or death. In PTCLTBX21, the median DFS was 41 months (standard error 21.50) and in PTCLGATA3 it was 12 months (standard error 6.2) with statistical significance (log rank test, p-value 0.04).

This was the first study in India, as per our literature search, that attempted the subclassification of PTCL-NOS using IHC. A greater sample size with more follow-up data would have added more credibility to the study. We have not used the markers granzyme B or TIA1 to look into the cytotoxic subset noted within the TBX21 subclass as this was beyond the scope of this study.

Conclusion

PTCL-NOS could be subclassified based on IHC staining of GATA3, CCR4, TBX21, and CXCR3 into PTCLGATA3 and PTCLTBX21 subtypes. PTCLGATA3 is a relatively aggressive disease showing a monomorphic morphology with a high ki67 index. The median DFS is lower in this subset with an increased occurrence of recurrence or relapse. In comparison, PTCLTBX21 showed a relatively lower ki67 levels with a polymorphous population and a higher median DFS. This could indicate that subcategorization of PTCL-NOS based on the IHC markers may help in the stratification of this disease. This will also identify cases with more aggressive behavior at the time of diagnosis.

Conflict of Interest
None declared.

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